STUDIES ON TRANSMETHYLATION AND CYSTINE SYNTHESIS IN SOME BACTERIA

BY

HELEN HIFT

B. S., Northwestern University, 1939

M. S., University of Illinois, 1945

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1948

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STUDIES ON TRANSMETHYLATION AND CYSTINE SYNTHESIS IN SOME BACTERIA

According to modern ideas the rat and other animals are able to synthesize cystine from dietary methionine. The pathway of this conversion seems to include the following steps (Binkley and du Vigneaud 1942): methionine is demethylated to homocysteine which is coupled with serine to form cystathionine which in the presence of ATP and zinc ions cleaves into phospho-homoserine and cysteine. A key position in this scheme has been accorded to homocystine which has been found to support the growth of young animals on a cystine-and methionine-free diet if choline was supplied (du Vigneaud, et al. 1939). Methionine, homocystine, and choline are, therefore, pictured as constituting a reservoire of labile methyl groups which are essential to the body for the synthesis of creatine, betaine and other similar compounds.

Since biochemical research in recent years has furnished evidence which would suggest a close parallelism between animal and microbial metabolism, the present study attempted to approach the problem of transmethylation and cystine and methionine synthesis in certain lactic acid bacteria from this point of view.

A review of the pertinent literature revealed much circumstantial evidence but few specific facts. A comparison of the nutritional requirements of bacteria and some analytical data regarding the protoplasmic contents in cystine, methionine, serine, choline, and ethanolamine would lead one to anticipate synthetic abilities of varying degrees in different bacterial species and strains. Work done on naturally and artificially produced mutants of *Escherichia coli* and related organisms and on the effect of anti-metabolites on nutrient requirements points toward anabolic capacities in the original strains. The fact that some bacteria are able to synthesize

creatine may also indicate the occurrence of transmethylation reactions in these organisms.

More specific studies regarding the sulfur requirements of various heterotrophic species would lead one to distinguish between cystine requirements which can also be met by other actual or potential sulfhydryl-compounds and more specific methionine and cystine demands. In some of the latter cases homocystine with or without choline has been found to substitute effectively for methionine (Starr 1946), but in the majority of the studies the results were negative. Much work has been done with *E. coli* mutants, which would suggest that the parent wild types were able to perform transmethylations (Lampen, et al. 1947).

In the present study *Lactobacillus arabinosus, Lactobacillus casei, Lactobacillus casei* var. *delbruckii, Leuconostoc mesenteroides* and *Streptococcus faecalis* strains were inoculated into a basal medium containing 16 amino acids with the exception of methionine and cystine and the extent to which growth occurred was determined titrimetrically after 72 hours incubation. It was found that methionine was non-essential and that cystine was greatly stimulating. In the case of *L. arabinosus* and *L. mesenteroides* this favorable response was also produced by cysteine, homocystine, and glutathione but not by thioglycollate, methyl mercaptan, and various other organic and inorganic sulfur-compounds. The two *L. casei* strains were helped by the presence of cysteine, homocystine, glutathione, thioglycollate and thiourea. The results with *Strep faecalis* did not give significant differences. When the experiments were repeated with a basal medium lacking in methionine, cystine, and serine, the first two organisms mentioned were still stimulated by cystine, cysteine, and glutathione but no longer by homocystine, while the two latter failed to develop under these conditions, indicating their absolute need of serine.

These results were interpreted as being indicative of a homocystine to cystine conversion via cystathionine in the

case of the *L. arabinosus* and *L. mesenteroides* strains studied. Similar conclusions could, however, not be reached in the case of the other three organisms on the basis of the data collected here.

An attempt was made to demonstrate transmethylation by growing bacteria in the presence of excess choline and ethanolamine and to determine the choline contents of their cell bodies by means of the *Neurospora* mutant method. *E. coli, L. arabinosus,* and *Strep. faecalis* were selected for this study and the basal medium contained hydrolyzed casein as a nitrogen source. The results, although not very satisfactory due to the great technical difficulties involved, seemed to indicate that ethanolamine was not converted into choline by the organisms studied.

On the whole the findings could not be interpreted as excluding the occurrence of transmethylation in these bacteria since other possible methyl donors and choline precursors were not tested. The explanation which was offered as the most likely was that of gene injury in the sense of Tatum and Beadle, thus rendering the organisms in question incapable of transforming methionine into homocysteine and of converting ethanolamine into choline.

REFERENCES

Binkley, F., and du Vigneaud, V., 1942. J. Biol. Chem. 144:507-511. The formation of cysteine from homocysteine and serine.

Du Vigneaud, V., Chandler, J. P., Moyer, A. W., and Keppel, D. M., 1939. J. Biol. Chem. 131:57-76. The effect of choline on the ability of homocystine to replace methionine in the diet.

Starr, M. P., 1946. J. Bact. 51:131-143. The nutrition of phytogenic bacteria. I. Minimal nutritive requirements of the genus *Xanthomonas.*

Lampen, J. O., Roepke, R. R., and Jones, M. J., 1947. Arch. Biochem. 13:55-66. Studies on the sulfur metabolism of *Escherichia coli*. III. Mutant strains of *E. coli* unable to utilize sulfate for their complete sulfur requirements.

VITA

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